

Amendments to the Specification:

With respect to the substitute specification submitted herewith, please revise the section at page 1, lines 2-4 to read as follows:

Related Application

This application is a divisional application of application Serial No. 09/355,664 filed October 8, 1999, which is the National Stage Entry of PCT/SE98/00277 filed February 17, 1998.

With respect to the substitute specification submitted herewith, please amend the paragraph at page 2, line 19 through page 3, line 15, to read as follows:

According to a first aspect, the present invention is directed to a modified extracellular domain of a cytokine receptor protein, capable of being crystallized without being complexed to a ligand molecule. These modified proteins substantially maintain their activity to their native ligands and they will therefore constitute powerful tools for ligand interaction studies. The inventive, modified cytokine receptor preferably is of the type which oligomerizes when being bound to a ligand. This may include heterooligomerization of homodimerization, as discussed in *Mol. Cell. Biol.*, 1994, Vol. 14(6), p.3535-49: S Watowich et al. Most preferably, the modified receptor is a homodimeric cytokine receptor, such as the growth hormone receptor (hGHR) having an extracellular part consisting of 237 amino acids in its native state. The inventive proteins have at least one molecule segment contributing to a disordered structure deleted. Preferably, the deletion results in a truncation in at least one terminal end and most preferably it is truncated both in its C-terminal end and in its N-terminal end. More preferably, the inventive proteins are modified human growth hormone receptors (hGHR) with 31 or 33 amino acid residues removed in its N-terminal end and/or with 3 or 4 amino acid residues removed in its C-terminal end. Even more preferably, the

inventive modified human growth hormone receptor (hGHR) consists of the amino acid residues 32-237 (SEQ ID NO: 2), 32-234 (SEQ ID NO: 3), or 34-233 (SEQ ID NO: 4) of the native molecule. Of these modified molecules, the truncated receptor consisting of amino acids 32-234 (SEQ ID NO: 3) of the native molecule is the most preferred. It should be emphasized that said modified cytokine receptors would be readily produced by the skilled person with existing methods of recombinant technology and their production in a recombinant host and their subsequent purification, therefore are not parts of the present invention. Further aspects of the invention are disclosed below.

With reference to the Substitute Specification submitted herewith, please amend the heading at page 3, line 16 as follows:

Brief Description of the Several Views of the Drawing.

With reference to the Substitute Specification submitted herewith, please amend the paragraph at page 3, lines 19-21 as follows:

Figs. ~~2a~~ 2A and ~~2b~~ 2B schematically demonstrate side and front views of a comparison of the crystal structures of free hGHR and hGHR interacting with hGH site 1 in the 1:2 complex; and

With reference to the Substitute Specification submitted herewith, please amend the paragraph at page 6, lines 13-21 as follows:

Figs ~~2a~~ 2A and ~~2b~~ 2B schematically demonstrate a comparison of the crystal structures of free hGHR and hGHR interacting with hGH site 1 in the 1:2 complex. In the structural alignment, only the C-terminal domains were used (residues 128-234) in order to clearly visualize the domain-domain movements upon transitions from free to complexed

form, wherein Fig. 2a 2A is a side view and Fig. 2b 2B is a front view. In the free receptor crystal, the two hGHR molecules in the asymmetric unit are very similar with a root mean square deviation of 0.18 Å comparing 184 Ca positions. The corresponding values for the liganded receptors in the 1:2 complex are 1.04 Å (181 Ca positions) and 1.17 Å (172 Ca positions) for the hGH site 1 and site 2 binding receptor respectively.

With reference to the Substitute Specification submitted herewith, please amend the paragraph at page 12, line 8 through page 14, line 2 to read as follows:

The hGH and hGHR used in the protein crystallographic work were expressed and purified as previously described in Sundström et al, (1996). Truncation mutants of hGHR were created using standard sub-cloning techniques and the expressed protein was assayed for hGH binding using affinity and size exclusion gel filtration chromatography as well as BIAcore (Pharmacia Biosensor, Sweden) measurements. The hGHR₃₂₋₂₃₄ (SEQ ID NO: 3) protein was crystallized by vapor diffusion, using 3 ml protein solution (7mg/ml in 10 mM ammonium acetate) mixed with 3 ml of 0.33 M NH₄SO₄ 30% (w/v) PEG-2000-dimethyl ether, 1% (v/v) DMSO and 100 mM MES buffer at pH 6.4 in a sealed tissue culture 24-well plate (Falcon, USA). The crystallization droplets were equilibrated at +18°C with 1 ml of the mother liquor for 2-4 weeks to obtain optimal quality crystals that diffracted to at least 2.9 Å with a conventional X-ray source. The crystals were frozen directly in the N₂ beam by adding a 1:1 mixture of 25% (v/v) ethylene glycol and 25% glycerol (v/v) to the crystallization droplet. Data was collected at station A1 at Cornell High Energy Synchrotron Source using a CCD detector (Area Detector Systems Corp., USA). The data was indexed, processed and scaled in the tetragonal spacegroup I4 using the programs DENZO and SCALEPACK, developed by S. Bailey in the SERC Daresbury Laboratory, Warrington, 1993. A molecular replacement search procedure was performed using the program

AMORE, also developed by Bailey, 1993. The co-ordinates of the site 1 binding hGHbp molecule in our 2.5 Å hGH:hGHR 1:2 complex was used. The highest scoring solution in the resolution interval 8 - 4 Å was found in space group I4₁ with two hGHbp molecules in the asymmetric unit. A rigid-body refinement in X-plor, described by J. Navaza in *Acta Cryst.*, 1994, Vol. A (50), pp. 157-163, with individual hGHbp domains including data between 10-6, 10-5 and 10-3.5 Å in each respective cycle, decreased both the R- and Free-R values (described by A.T. Brünger in *Nature*, 1992, Vol. 355, pp. 472-475) dramatically when compared to previous runs where the native hGHbp domain arrangement was used. A cyclic process of model building in O, described by Brünger, 1992, followed by NCS restrained POWELL minimization in X-plor, using data between 15 - 2.3 Å, which was corrected for most main and side chain changes to the search molecule. At this stage, the first simulated annealing run, as described by T.A. Jones, et al. in *Acta Cryst*, 1991, Vol. A(47), pp. 110-119, was performed using a slow-cooling protocol from 3000 K to 300K in 50 Ps steps. Solvent molecules were introduced into FoFc densities above 3.0 s. After 3 cycles, a total of 327 solvent molecules had been introduced and assigned to the protein chain using the programs DISTANG and WATERTIDY developed by A.T. Brünger et al., 1989, in the CCP4 program package. A final POWELL minimization was performed, followed by a simulated annealing run from 2500 K to 300 K in 50 ps steps and including data between 15 to 2.3 Å. Individual B-value refinement was added as the final step, and solvent molecules with high temperature factors, greater than 50 Å², or absent 2FoFc electron densities cut-off at 1.0 s, were removed. The Free-R value was used to validate the progress of the entire refinement. The final model consisted of residues 32 - 52, 63 - 70 and 80 - 234 of both molecules in the asymmetric unit as well as 261 solvent molecules and two sulphate ions. At the present stage of refinement, the R-factor of the model is 21.7% (R-free 29.3%), using data between 10 - 2.3 Å. As a control, a dataset to 3.2 Å at room temperature was collected. No significant

differences to the 2.3 Å structure were observed, showing that the transfer to cryogenic conditions did not induce conformational adaptation. See also, Merritt et al, *Acta. Cryst.*, D50, 869-73 (1994).

With respect to the Substitute Specification submitted herewith, please amend the Table 1 heading at page 15, line 3 to read as follows:

Crystallographic data for hGHR₃₂₋₂₃₄ (SEQ ID NO: 3).